INVESTIGATION TO INCREASE THE VIRICIDAL CAPACITY OF DISINFECTANT, GERMICIDAL AND FUNGICIDAL, PHENOLIC, DRY-TYPE

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The straight O-D-1435 Type II disinfectant had little capacity to inactivate the poliovirus type I when used in concentrations up to 15,000 mg/l with contact times up to 24 hours. The Coxsackie A9 and Echo 12 viruses showed no inactivation with 10,000 mg/l of disinfectant at contact times of 30 minutes. An increase of the pH to more than 12 did potentiate the disinfectant for poliovirus inactivation. The survival at a pH of 12.1 with 10,000 mg/l of disinfectant was less than 0.01 percent after 5 minutes of contact. The survival at

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this pH without the disinfectant was 58 percent. Anionic surfactants in concentrations from 100 to 10,000 mg/l had little capacity for increasing the viricidal capacity of the disinfectant. Cationic and nonionic surfactants had no enhancement ability.

FINAL REPORT

on

Investigation to Increase the Viricidal Capacity of Disinfectant, Germicidal and Fungicidal, Phenolic, Dry Type

Contract DAAG-17-73-C-0190
3 May 1973 - 28 February 1975

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February 28, 1975

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FINAL REPORT

on

Investigation to Increase the Viricidal Capacity of Disinfectant,

Germicidal and Fungicidal, Phenolic, Dry Type

This is the final report of progress on the project on the potentiation of the military disinfectant, Federal Specification O-D-1435 Type II (Lot 7A, pkg 7/67, from Chemical Compounding Corp., Jersey City, N.J.), for virus inactivation, contract number DAAG-17-73-C-0190. This report presents the data obtained from the studies required under the terms of the contract plus additional work done at no additional cost to the government. This latter work was done as a result of discussions during the course of the project between the local project director and the responsible contract officers at the U.S. Army Natick Laboratories.

GENERAL METHODOLOGY

VIRUS CULTURE

Poliovirus type I (Mahoney) was used in all of the experimental work. The virus was cultured by serial passage in LLC MK_2 (Hull) Rhesus monkey kidney cells. The stock virus was subjected to three freeze-thaw cycles and then clarified by low speed centrifugation. The suspension was dialyzed against polyethylene glycol and lipids and nonviral protein removed by homogenizing the suspension with a fluorcarbon (Genetron 113). This emulsion was broken by centrifugation at about 1,000 times gravity. The aqueous suspension containing the poliovirus was decanted and had a virus titer of about 10^8 PFU/ml. The virus suspension was kept frozen until used.

VIRUS ENUMERATION

The virus titering was done with LLC MK_2 cells using the tissue culture technique of Dulbecco and Vogt (1) and Vogt et al. (2). The plates were normally counted on days 5, 6 and 7.

Coxsackie A9 and Echovirus 12 were also grown out by the techniques above, prepared for use and tested for inactivation with the straight disinfectant. All of the active experimental work was done with the poliovirus, however.

Determination of the titer was done using two or three dilutions of the sample and three plates were prepared at each dilution.

EXPERIMENTAL PROCEDURES

The dilution water used for all of the work was triple distilled water with 100 mg/l of added NaCl. The temperature for all of the work was 22°C with several of the experiments done at 23°C. The initial poliovirus titer for all of the work was about 10^5 PFU/ml. The experimental data represent the averaged results from duplicate experiments unless otherwise indicated. All glassware was treated with a hot Haemosol detergent solution to minimize virus adsorption to the glassware.

The experiments were carried out with the following typical conditions:

(1) One thousand ml beakers with a final volume of 750 ml of the reacting solutions were used. One of the beakers containing only the NaCl and the virus was used as a control. The remaining beakers contained the appropriate amounts of the reactants.

(2) The necessary amount of stock virus solution was added to each beaker and mixed under a laboratory stirring machine for 30 seconds at 80 RPM. The zero time sample was then taken from the control beaker. During the portion of the work with elevated pH the beakers were covered with plastic and nitrogen gas was purged under the plastic to prevent adsorption of CO_2 from the atmosphere and consequent lowering of the pH.

(3) Samples were withdrawn for titering from all beakers with reacting solutions after various contact times. Results were expressed as the percent change from the titer in the control. The control was also sampled at the conclusion of the experiment.

EXPERIMENTAL RESULTS

ANALYSIS OF DISINFECTANT

The O-D-1435 Type II military disinfectant used in this project was analyzed for moisture and composition. The analyses were performed in general conformity with the procedures contained in Federal Specification O-D-1435A dated August 25, 1971 (3). The disinfectant used was Lot 7A, pkg 7/67 manufactured by the Chemical Compounding Corp., Jersey City, New Jersey. P-chlorophenol was used as an internal standard for the analyses.

The results of the analyses and the instrument conditions are presented in Table 1. The percentage compositions were all within the ranges specified in the Federal Specification. This particular lot contained 6.88 percent of material which was not determined by the analyses. The lot of disinfectant which was used met the Federal Specification.

INACTIVATION WITH THE STRAIGHT DISINFECTANT

Table 2 presents the data obtained where the disinfectant was used without pH adjustment beyond the ph which occurred from the buffers in the disinfectant. These data show that a small amount of inactivation was achieved by the disinfectant. The inactivation was only about 10 percent at 30 minutes for the poliovirus and did not appear to increase significantly as the disinfectant was increased from 2,000 to 10,000 mg/l. Essentially no inactivation was obtained with the Coxsackie A9 and Echo 12 viruses over the 30 minute observation period.

Table 3 presents the data from an inactivation study with the straight disinfectant with a contact time of 24 hours. Disinfectant concentrations as high as 15,000 mg/l produced an increase of only about 10 percent more inactivation than that which occurred by natural dieoff in the water suspension system which was used. Potentiation of the disinfectant will be required if it is to inactivate viruses.

INACTIVATION WITH pH ADJUSTMENT

The data obtained for the virus inactivation with the disinfectant at pH values of 11.5, 11.9, 12.1 and 12.5 are shown in Tables 4, 5, 6 and 7. The pH was increased by adding appropriate quantities of NaOH. The inactivation obtained in a control sample held at the pH of the experiment but without added disinfectant is also shown in each Table. In examining the inactivation obtained in the controls without added disinfectant it may be noted that the survivals decreased from 97 percent at 5 minutes at pH 11.5 to 0.17 percent at pH 12.5.

The data in Tables 4 through 7 for the 2,000 mg/l disinfectant concentration did not show significant improvement in the inactivation over that obtained by the pH alone as shown in the control experiments. As an example, in Table 6 the survivals in the control experiment at pH 12.1 differ by only a few percent from those obtained at the 2,000 mg/l disinfectant concentration after 5 minutes or more of contact time.

The 10,000 mg/l disinfectant concentration data show significantly increased inactivations over that from the pH alone. The inactivation results at 10,000 mg/l of disinfectant at each of the pH values which are plotted in Figure 1. The data for pH 11.2 was taken from Table 2.

TABLE 1
GAS CHROMATOGRAPHIC ANALYSIS
OF THE DISINFECTANT

Component	Amount Present %
Moisture:	14.83
Sodium 4-Cl-2-phenylphenolate:	41.88
Sodium 6-Cl-2-phenylphenolate:	14.05
Sodium orthophenylphenolate:	22.36

Instrument Conditions

Instrument:	F & M Chromatograph Model 402
Flash Heater:	300°C
Detector:	250°C
Column:	210°C
Helium:	80 ml/min @ 80 psi
Chart Speed:	0.5 inches/min
Range:	10^3
Attenuation:	32
Sample size:	10 microliters

TABLE 2
INACTIVATION OF POLIOVIRUS I, COXSACKIE A9
AND ECHOVIRUS 12 BY THE STRAIGHT
DISINFECTANT

Disinfectant Concentration mg/l	pH	Percent Survival Time of Contact Minutes					
		0	5	10	20	30	90
POLIOVIRUS I*							
2,000	10.75	100	104	103	97	89	70
4,000	10.90	100	107	96	100	86	68
10,000	11.21	100	98	85	83	91	79
COXSACKIE A9**							
2,000	10.81	100	100	105	127	111	
4,000	10.96	100	120	90	105	108	
10,000	11.14	100	94	139	111	114	
ECHO**							
2,000	10.76	100	103	99	107	119	
4,000	10.98	100	100	112	104	105	
10,000	11.26	100	106	95	123	91	

* Initial titer - 9.3×10^4 PFU/ml
** Initial titer - 1.5×10^5 PFU/ml

TABLE 3
INACTIVATION OF POLIOVIRUS I WITH
A 24 HOUR CONTACT WITH
THE STRAIGHT DISINFECTANT

Disinfectant Concentration mg/l	pH	Percent Survival* Time of Contact Hours				
		0	0.5	3	8	24
0 (Control)	7.0	100	90	80	53	24
4,000	11.00	100	91	57	56	13
10,000	11.34	100	99	48	42	12
15,000	11.51	100	89	43	31	10

* Average of three determinations

Initial titer - 2×10^5 PFU/ml

TABLE 4

INACTIVATION OF POLIOVIRUS I WITH DISINFECTANT AT pH 11.5

Disinfectant Concentration mg/l	Percent Survival* Time of Contact Minutes				
	0	5	10	20	30
None (Control at pH 11.5)	100	97	117	86	93
2,000	100	115	128	96	95
4,000	100	102	107	80	69
10,000	100	77	88	68	52

* Average of three determinations

Average initial titer 2.5×10^5 PFU/ml

TABLE 5

INACTIVATION OF POLIOVIRUS I WITH DISINFECTANT AT pH 11.9

Disinfectant Concentration mg/l	Percent Survival Time of Contact Minutes				
	0	5	10	20	30
None (Control at pH 11.9)	100	100	66	53	41
2,000	100	121	85	72	49
4,000	100	81	75	51	45
10,000	100	47	38	23	NM

NM - Not measured

Average initial titer 1.3×10^5 PFU/ml

TABLE 6

INACTIVATION OF POLIOVIRUS I WITH DISINFECTANT AT pH 12.1

Disinfectant Concentration mg/l	Percent Survival Time of Contact Minutes				
	0	5	10	15	20
None (Control at pH 12.1)	100	58	10	4	6
2,000	100	41	7	5	4
4,000	100	4	0.4	<0.01	<0.01
10,000	100	<0.01	0.07	<0.01	<0.01

Average initial titer 2.4×10^5 PFU/ml

TABLE 7

INACTIVATION OF POLIOVIRUS I WITH DISINFECTANT AT pH 12.5

Disinfectant Concentration mg/l	Percent Survival Time of Contact Minutes				
	0	0.5	1	3	5
None (Control at pH 12.5)	100	1.5	0.4	0.2	0.17
2,000	100	0.28	0.21	0.12	0.05
4,000	100	1.0	0.2	<0.01	<0.01
10,000	100	0.27	0.27	0.4	<0.01

Average initial titer 3.3×10^5 PFU/ml

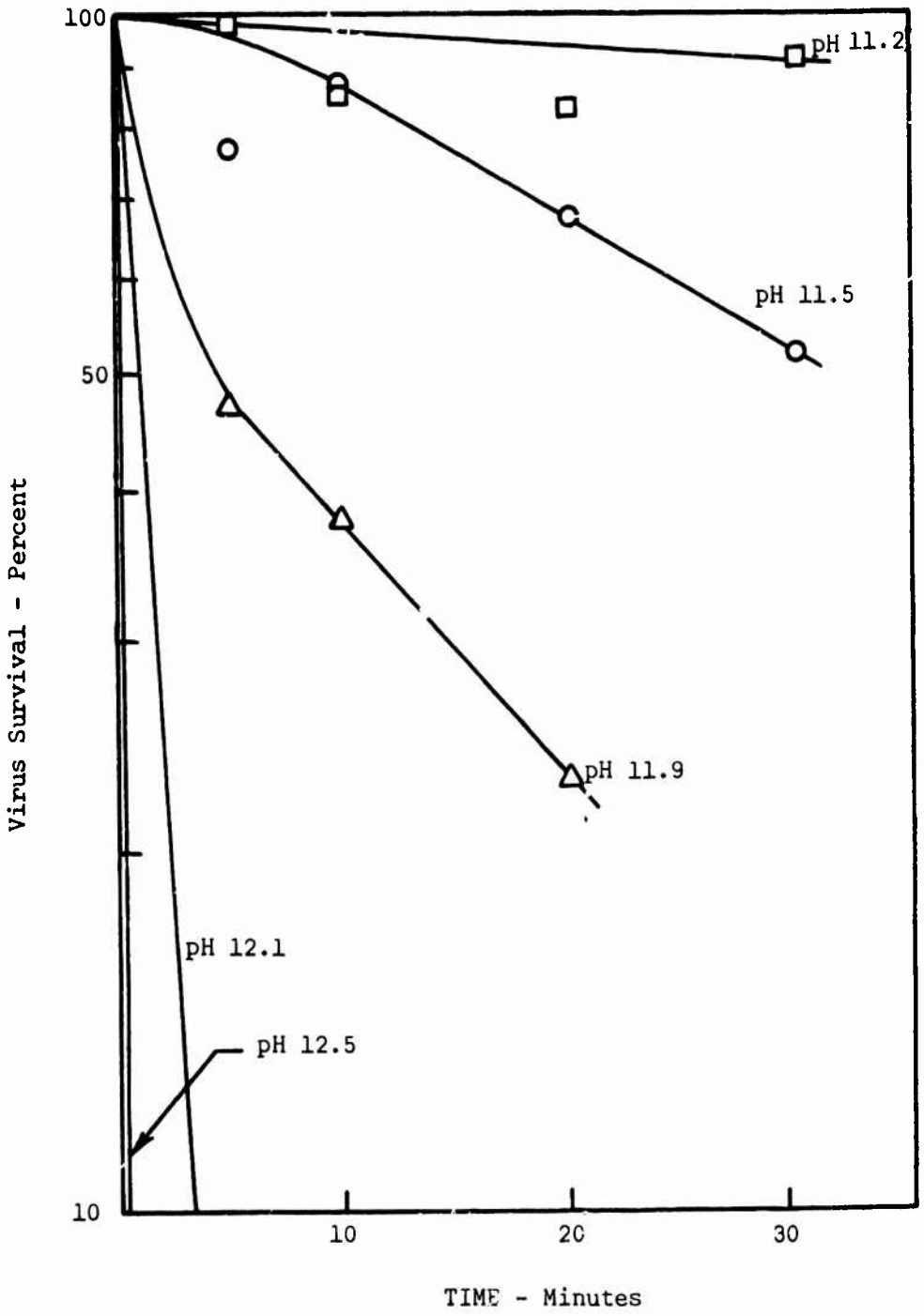

Figure 1. Poliovirus I Inactivation With 10,000 mg/l of Disinfectant At Various pHs.

At a pH of 12.5, shown in Table 7, this increased inactivation effect is still noticeable but is masked somewhat by the high inactivation from the pH alone.

The data in Tables 2, 4, 5, 6 and 7 for virus survival at 4,000 mg/l of disinfectant have been plotted in Figure 2. The plots show that the inactivation effect from the disinfectant is noted at this concentration as the pH is increased from 10.9 to 12.5.

Summary

These results show that the inactivation of the poliovirus I with the phenolic disinfectant can be increased when the pH is raised to values up to 12.5. The total increase in the inactivation is caused by a pH effect on the virus and by a toxic effect from the chlorophenolic disinfectant. The toxic effect from the disinfectant appears more pronounced at higher pH values since the concentration of disinfectant at which the effect is noted is lowered. Thus the inactivation appears to have been improved at 10,000 mg/l at a pH of 11.5 (Table 4) but the improvement is noted at 2,000 mg/l at pH 12.5 (Table 7). This toxic effect from the disinfectant is not, however, particularly pronounced. The major portion of the increased inactivation at the elevated pH values is attributed to the pH effect alone.

INACTIVATION WITH SURFACTANTS

Surfactants Selected For Investigation

The surfactants used in this investigation are shown in Table 8. These surfactants include those selected for inclusion by the Project Officer, Dr. Arthur M. Kaplan, and those selected by the Principal Investigator, Dr. Otis J. Sproul. The surfactants were selected after consulting the literature and after discussion with surfactant specialists from the Shell Chemical Company, Diamond Shamrock Company, Monsanto Chemical Company, Richardson Company, Proctor and Gamble Company and the Dow Chemical Company. This list is representative of surfactants with respect to their chemical structure and surface charge which are potentially useful for potentiation of the disinfectant.

Research Methodology

The screening work for the surfactants was carried out with the poliovirus I with the disinfectant at a concentration of 4,000 mg/l and a 30 minute contact time. The 4,000 mg/l concentration was chosen since the work presented in Table 2 indicated that the poliovirus I was just on the edge of showing inactivation at the 4,000 mg/l concentration of disinfectant after 30 minutes of contact. Significant enhancement of

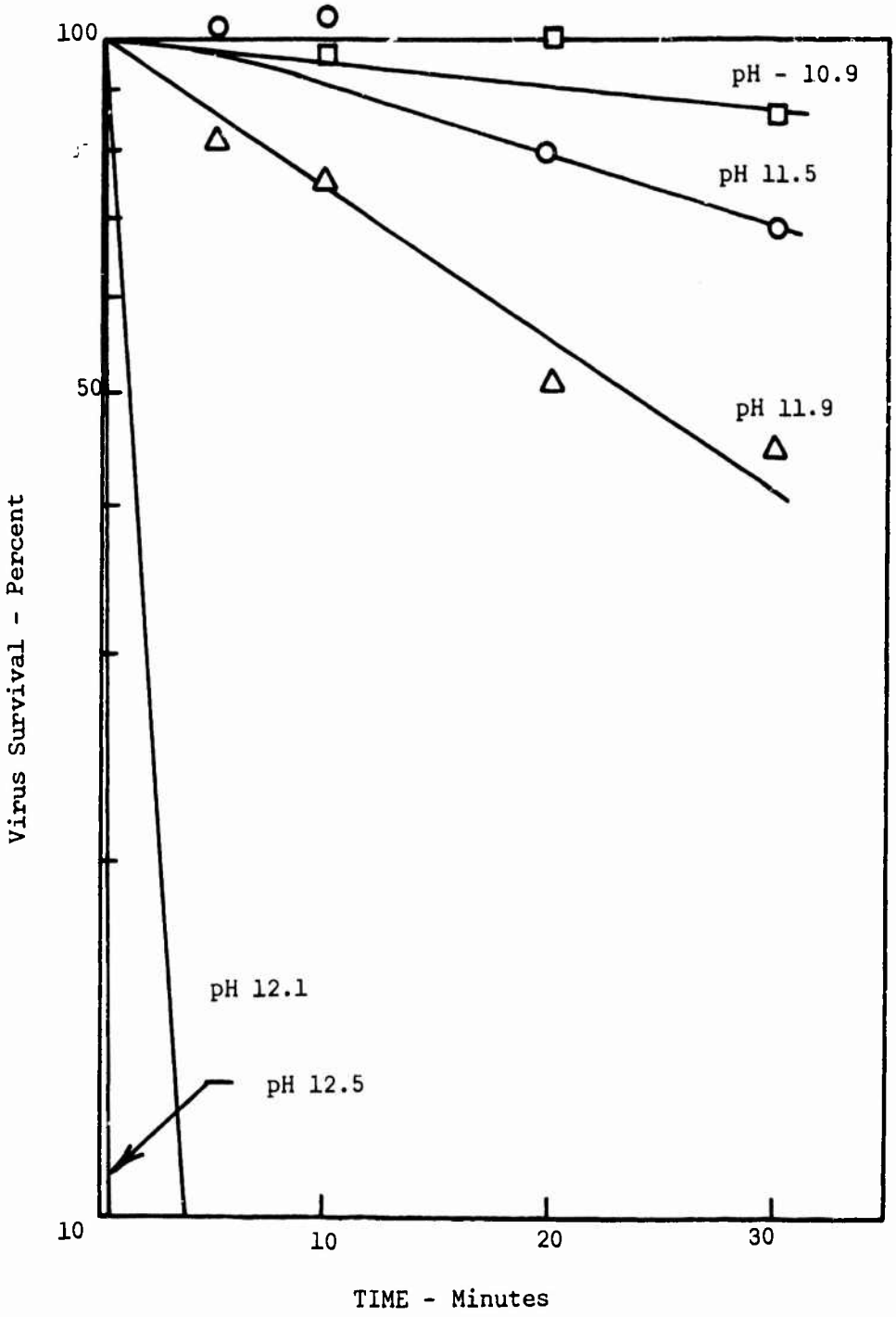

Figure 2. Poliovirus I Inactivation With 4,000 mg/l of Disinfectant At Various pHs.

TABLE 8

SURFACTANTS SELECTED FOR INVESTIGATION

Brand Name and Manufacturer	Surface Charge	Chemical Structure
Neodol 25-3S Shell Chemical Co.	Anionic	C_{12} - C_{15} Alcohol (3EO) ethoxysulfate, Sodium salt
Neodol 23 - 6.5 Shell Chemical Co.	Nonionic	C_{12} - C_{13} Alcohol (6.5 EO) Ethoxylate
Neodol 25 - 9 Shell Chemical Co.	Nonionic	C_{12} - C_{15} Alcohol (9 EO) Ethoxylate
Dowfax 2A1 Dow Chemical Co.	Anionic	Disodium - 4 - dodecylated oxydibenzene sulfonate
Dowfax 3B2 Dow Chemical Co.	Anionic	Disodium - 4 - decylated oxydibenzene sulfonate
XD - 8214 Dow Chemical Co.	Anionic	A new experimental surfactant recommended by Dow's Surfactant Group. Similar to Dowfax 3B2 but with less sulfonation.
Hyonic PE90 Nopco Chemical Div., Diamond Shamrock Chemical Co.	Nonionic	Polyethyoxylated (9 EO) nonylphenol

TABLE 8 CONTINUED

SURFACTANTS SELECTED FOR INVESTIGATION

Brand Name and Manufacturer	Surface Charge	Chemical Structure
Commercial sodium stearate Nopco Chemical Div., Diamond Shamrock Chemical Co.	Anionic	Sodium salt of stearic acid
Commercial sodium oleate Nopco Chemical Div., Diamond Shamrock Chemical Co.	Anionic	Sodium salt of oleic acid
Richonate 1850 Richardson Co.	Anionic	Sodium alkylaryl sulfonate
Monflor 71 Imperial Chemical Industries, Ltd., (England)	Cationic	Fluoro organic compound
Cold Power Colgate-Palmolive Co.	Anioric	Sodium alkylbenzene sulfonate (household clothes detergent)

the inactivation by any of the surfactants would become obvious under these conditions.

The surfactants were screen tested at concentrations of 100 and 1,000 mg/l unless evidence was obtained which indicated that a lower concentration was desirable. In some cases the solubility of the surfactant limited the upper concentration level. Additionally, precipitation of the disinfectant resulted when cationic or nonionic surfactants in higher concentrations were used. This reduced the effective concentrations of the surfactant and disinfectant which remained in solution.

Surfactant Toxicity To Monkey Cells

Preliminary testing with Monflor 71 and Cold Power was carried out to determine the toxicity of these materials to the LLC MK_2 monkey cells and to furnish background data on how the other surfactants might be expected to react with the cells. The data are presented in Table 9. The procedure used was to mix the indicated concentration of the surfactant in Earles BSS without bicarbonate. The usual volume taken for titering (0.2 ml) was added to each of 5 plates and incubated for 30 minutes at 37°C. The liquid was drawn off and overlayed with normal agar overlay medium. The cells were examined after 2 or 3, 4 and 5 days of incubation to determine the amount of cell sheet destruction.

The results presented in Table 9 show that the Cold Power and Monflor 71 were toxic to kidney cells at concentrations of 1 mg/l or more applied directly to the cells. A concentration of 1 mg/l or more was not applied directly to the cell sheet in subsequent work since the amount of inactivation which was obtained required dilutions of the reacting suspensions which reduced the surfactant concentrations to less than 1 mg/l.

Inactivation With Monflor 71 and Disinfectant

The data for the inactivation of the poliovirus I with the disinfectant and Monflor 71 are presented in Table 10. The data for the Monflor 71 without added disinfectant show that Monflor 71 itself, in concentrations up to 1,000 mg/l, was not toxic to the virus. Survivals were all about 100 percent after 30 minutes of contact with the surfactant. It should be noted that the pH of the suspension decreased when Monflor 71 was added reaching 3.33 at 1,000 mg/l.

The Monflor 71 reacted with the 4,000 mg/l of disinfectant to produce a turbid suspension at surfactant concentrations of 10, 100 and 1,000 mg/l. The turbidity increased as the surfactant concentration increased indicating more precipitation and a reduced concentration of the disinfectant in solution at the higher concentrations.

TABLE 9
TOXICITY OF TWO SURFACTANTS TO
LLC MK_2 MONKEY KIDNEY CELLS

Surfactant Concentration mg/l	Destruction of Cell Sheet on Each of Five Plates Days of Incubation			
	2 %	3 %	4 %	5 %
MONFLOR 71				
1000		60	60	60
100		20	20	20
10		10	10	10
1		0	0	0
.1		0	0	0
.01		0	0	0
.001		0	0	0
0 (Control)		0	0	0
COLD POWER				
100	20	-	20	20
10	10	-	10	10
1	0	-	0	0
.1	0	-	0	0
.01	0	-	0	0
0 (Control)	0	-	0	0

TABLE 10

INACTIVATION OF POLIOVIRUS I WITH DISINFECTANT AND MONFLOR 71

Sample Disinfectant mg/l	Monflor 71 mg/l	pH	Percent Survival* Time of Contact Minutes 0	5	10	20	30
0 (Control)	0	7.35	100				100
0	.001	6.35	100	92	109	108	75
0	.01	6.33	100	100	100	80	100
0	.05	6.21	100	114	66	Mold	107
4,000	.001	10.98	100	100	69	117	125
4,000	.01	10.98	100	-	117	92	125
4,000	.05	10.95	100	114	100	114	121
0	10	5.73	100	105	106	106	110
0	100	4.40	100	94	118	126	88
0	1,000	3.33	100	93	88	102	100
4,000	10	10.95	100	107	105	93	90
4,000	100	10.90	100	104	75	94	80
4,000	1,000	10.86	100	95	85	87	120

* Data are for one determination through .05 mg/l of Monflor, duplicate determinations for 10 & 100 mg/l and triplicates for 100 and 1,000 mg/l.

The Monflor 71 plus 4,000 mg/l of disinfectant did not produce significant inactivation effects through the .001 to 1,000 mg/l of concentrations which were used. Some decrease in titer was observed at the 10, 100 and 1,000 mg/l concentration ranges but it was not deemed significant. It was also felt that this decrease may have been caused in part by adsorption of the virus to the precipitate which was formed. The use of higher concentrations did not seem warranted since the precipitation effect would become more pronounced at these concentrations.

Inactivation With Cold Power And Disinfectant

The data for Cold Power and the disinfectant are shown in Table 11. The data show no toxicity to the poliovirus I when straight Cold Power in concentrations of 100 and 1,000 mg/l was present nor any inactivation when the disinfectant was present in concentrations of 4,000 mg/l along with the Cold Power.

Inactivation With Richonate 1850 And Disinfectant

The data for the inactivation of the poliovirus with Richonate 1850, an alkylaryl sulfonate, at concentrations of 100 and 1,000 mg/l are shown in Table 12. The data do not show any direct toxicity to the virus with the Richonate 1850 nor any potentiation of the disinfectant.

Inactivation With Sodium Stearate, Sodium Oleate and Disinfectant

Sodium stearate has limited solubility in water with low inorganic salt content and at room temperature. The experimental procedures used in this work used the virus in a diluting medium with 100 mg/l of NaCl and a temperature of 20°C. These conditions were such as to lead to a limited solubility of sodium stearate. Manufacturer's recommendations indicated that the sodium stearate might be dispersed as a collodial sol in our water medium by heating the liquid, mixing in the sodium stearate and then cooling to the operating temperature. Consequently the diluting medium with the sodium stearate, but minus the virus was heated to 75°C with several stearate concentrations over the range from 100 mg/l to 1,000 mg/l. Upon cooling a heavy, gelatinous sol formed at all concentrations in excess of about 100 mg/l. Even at this concentration a very fine precipitate did form indicating a sol formation. Further preliminary work at 100 mg/l of sodium stearate indicated that a flocculent precipitate formed when 4,000 mg/l of the disinfectant was added.

In spite of these difficulties, and with these limitations, an experiment was carried out at 100 mg/l of sodium stearate to determine its effect upon the disinfectant-virus system. The data presented in Table

TABLE 11

INACTIVATION OF POLIOVIRUS I WITH
DISINFECTANT AND COLD POWER

Sample			Percent Survival				
Disinfectant	Cold Power		Time of Contact Minutes				
mg/l	mg/l	pH	0	5	10	20	30
0 (Control)	0	7.14	100	-	-	-	102
0	100	9.15	100	90	77	89	91
0	1,000	9.85	100	100	87	91	106
4,000	100	10.83	100	113	108	100	109
4,000	1,000	10.89	100	102	102	117	109

TABLE 12

INACTIVATION OF POLIOVIRUS I WITH
DISINFECTANT AND RICHONATE 1850

Sample			Percent Survival				
Disinfectant	Richonate 1850		Time of Contact				
mg/l	mg/l	pH	Minutes				
			0	5	10	20	30
Data for 100 mg/l of Richonate 1850							
0	0	7.58	100				106
0	100	6.37	93				106
4,000	100	10.84	107				86
Data for 1000 mg/l of Richonate 1850							
0	0	7.05	100				116
0	1,000	6.57	100	103	97	100	101
4,000	1,000	11.00	100	100	103	90	108

13 show a minor inactivation of 26 percent in 30 minutes by the sodium stearate alone and about 36 percent inactivation by the sodium stearate plus the disinfectant. It was not felt that these inactivations were of sufficient magnitude to be taken as significant potentiation.

Sodium oleate is a component of commercial soaps and because of its unsaturation it is more soluble. It was used in a concentration of 1,000 mg/l with the disinfectant yielding the results in Table 14. The oleate in the concentration used here did not show any inactivation potential, either alone or in combination with the disinfectant.

Inactivation with Dowfax 2A1 and Disinfectant

Dowfax 2A1 (disodium-4-dodecylated oxydibenzene sulfonate) was investigated with the results shown in Table 15. The data do not show any potentiation of the disinfectant nor does the surfactant itself appear to cause any inactivation of the poliovirus.

Inactivation with Dowfax XD-8214 and Disinfectant

Dowfax XD-8214, a new experimental anionic surfactant from Dow Chemical Company, was investigated at concentrations of 1,000 and 10,000 mg/l. Dowfax XD-8214 is similar to the Dowfax 2A1 except that the aliphatic side chain has been shortened. The data from these experiments are presented in Table 16. The Dowfax XD-8214 at a concentration of 1,000 mg/l caused a decrease of 34 percent in the titer of the poliovirus I. Additional inactivation was not obtained when the disinfectant was added, however. The surfactant at a concentration of 10,000 mg/l, however, did not cause any change in the titer of the poliovirus when used alone or when used with the disinfectant. The reasons for this apparent protective behavior at the higher concentration of disinfectant are not clear.

Inactivation with Dowfax 3B2 and Disinfectant

Dowfax 3B2, an anionic surfactant, was used in concentrations of 100 and 1,000 mg/l. The data, presented in Table 17, do not show any potentiation of the disinfectant.

Inactivation with Hyonic PE-90 and Disinfectant

Hyonic PE-90, a polyethoxylated nonylphenol, has been investigated in a concentrations of 100 and 1,000 mg/l. The data for this work are

TABLE 13

INACTIVATION OF POLIOVIRUS I WITH
DISINFECTANT AND SODIUM STEARATE

Sample							
Disinfectant	Sodium Stearate		Percent Survival				
			Time of Contact				
mg/l	mg/l	pH	Minutes				
			0	5	10	20	30
0 (Control)	0	7.63	100				92
0	100	9.53	100	97	98	106	74
4,000	100	10.85	100	114	103	97	64

TABLE 14

INACTIVATION OF POLIOVIRUS I WITH
DISINFECTANT AND SODIUM OLEATE

Sample			Percent Survival				
Disinfectant	Sodium Oleate		Time of Contact Minutes				
mg/l	mg/l	pH	0	5	10	20	30
0 (Control)	0	7.20	100				82
0	1,000	9.80	100	99	90	115	105
4,000	1,000	10.90	100	97	105	107	100

TABLE 15

INACTIVATION OF POLIOVIRUS I WITH
DISINFECTANT AND DOWFAX 2A1

Sample			Percent Survival				
Disinfectant	Dowfax 2A1		Time of Contact Minutes				
mg/l	mg/l	pH	0	5	10	20	30
Data for 100 mg/l of Dowfax 2A1							
0	0	7.30	100				100
0	100	7.31	100	96	96	94	93
4,000	100	11.05	100	105	105	105	92
Data for 1000 mg/l of Dowfax 2A1							
0	0	7.40	100				83
0	1,000	7.56	100	93	104	119	97
4,000	1,000	10.86	100	91	101	106	103

TABLE 16

INACTIVATION OF POLIOVIRUS I WITH
DISINFECTANT AND DOWFAX XD-8214

Disinfectant	Dowfax XD-8214		Percent Survival Time of Contact Minutes				
mg/l	mg/l	pH	0	5	10	20	30
0 (Control)		6.60	100				100
0	1,000	8.00	100	102	113	104	66
0	10,000	9.40	100	105	93	97	103
4,000	1,000	10.88	100	98	102	74	72
4,000	10,000	11.33	100	95	99	106	99

TABLE 17

INACTIVATION OF POLIOVIRUS I WITH DISINFECTANT AND DOWFAX 3B2

Sample			Percent Survival				
Disinfectant	Dowfax 3B2		Time of Contact				
mg/l	mg/l	pH	Minutes				
			0	5	10	20	30
Data for 100 mg/l of Dowfax 3B2							
0	0	7.43	100				121
0	100	6.85	100	108	108	110	106
4,000	100	10.95	100	96	114	100	90
Data for 1000 mg/l of Dowfax 3B2							
0	0	7.65	100				100
0	1,000	6.85	100	98	97	96	97
4,000	1,000	10.96	100	118	107	107	106

shown in Table 18. The Hyonic PE-90 did not show any ability to inactivate the virus either alone or when used with the disinfectant.

Inactivation with Neodol 25-3S and Disinfectant

The data for the inactivation of the poliovirus with Neodol 25-3S, an anionic surfactant (sodium salt of a C_{12} - C_{13} alcohol (3EO) ethoxysulfate) are presented in Table 19. The data suggest that this anionic may have been useful in causing some small potentiation of the disinfectant since a 30 percent inactivation was obtained at 1,000 mg/l of the surfactant and 4,000 mg/l of the disinfectant. This was 22 percent more inactivation than that obtained with the surfactant alone.

Inactivation With Neodol 23-6.5 and Neodol 25-9 and Disinfectant

Neodol 23-6.5 and Neodol 25-9 will be considered together since they are nonionic surfactants with similar structure. The Neodol 23-6.5 has 6.5 ethylene oxides per alcohol (mol/mol) which the Neodol 25-9 has 9 ethylene oxides per alcohol. The Neodol 25-9 formed a precipitate when the disinfectant was added. The data presented in Tables 20 and 21 do not show any inactivation potential for these surfactants.

INACTIVATION WITH TERGISYL

An inactivation experiment using premeasured Tergisyl and poliovirus was run at the suggestion of Dr. Arthur M. Kaplan, the Project Officer. Tergisyl is produced by National Laboratories, Lehn & Fink Industrial Products Division, Sterling Drugs Inc., Montvale, N.J. Premeasured Tergisyl is a chlorophenate disinfectant with the following ingredients:

	Percent Present
K - o - Benzyl - p - Chlorophenate	7.57
Tetrasodium Ethylene Diamine Tetraacetate	2.0
Wetting agents, detergents, inert ingredients	90.43

The recommended dosage of one ounce per gallon was used in the experiments. The data are presented in Table 22. The data did not show any inactivation of the poliovirus by the Tergisyl.

TABLE 18

INACTIVATION OF POLIOVIRUS I WITH
DISINFECTANT AND HYONIC PE-90

Sample							
Disinfectant	Hyonic PE-90		Percent Survival				
			Time of Contact				
mg/l	mg/l	pH	Minutes				
			0	5	10	20	30
Data for 100 mg/l of Hyonic PE-90							
0	0	7.32	100				100
0	100	6.21	100	102	112	97	106
4,000	100	10.66	100	98	97	107	102
Data for 1000 mg/l of Hyonic PE-90							
0	0	7.30	100				94
0	1,000	5.84	100	119	113	95	91
4,000	1,000	11.40	100	105	125	109	114

TABLE 19

INACTIVATION OF POLIOVIRUS I WITH
DISINFECTANT AND NEODOL 25-3S

Sample			Percent Survival				
Disinfectant	Neodol 25-3S		Time of Contact Minutes				
mg/l	mg/l	pH	0	5	10	20	30
Data for 100 mg/l of Neodol 25-3S							
0	0	7.40	100				100
0	100	6.91	100	101	98	104	110
4,000	100	10.90	100	94	94	109	84
Data for 1000 mg/l of Neodol 25-3S							
0	0	7.31	100				104
0	1,000	6.23	100	100	104	97	92
4,000	1,000	11.04	100	100	95	86	70

TABLE 20

INACTIVATION OF POLIOVIRUS I WITH
DISINFECTANT AND NEODOL 23-6.5

Sample							
Disinfectant	Neodol 23-6.5		Percent Survival				
			Time of Contact				
mg/l	mg/l	pH	Minutes				
			0	5	10	20	30
Data for 100 mg/l of Neodol 23-6.5							
0	0	7.58	100				98
0	100	6.47	100	97	98	100	104
4,000	100	10.91	100	96	100	108	84
Data for 1000 mg/l of Neodol 23-6.5							
0	0	7.32	100				119
0	1,000	6.61	100	105	108	110	108
4,000	1,000	11.31	100	102	100	112	109

TABLE 21

INACTIVATION OF POLIOVIRUS I WITH DISINFECTANT AND NEODOL 25-9

Sample			Percent Survival				
Disinfectant	Neodol 25-9		Time of Contact Minutes				
mg/l	mg/l	pH	0	5	10	20	30
Data for 100 mg/l of Neodol 25-9							
0	0	6.70	100				104
0	100	6.45	100	100	106	101	90
4,000	100	11.04	100	118	84	107	100
Data for 1000 mg/l of Neodol 25-9							
0	0	7.25	100				96
0	1,000	6.64	100	96	100	102	112
4,000	1,000	11.31	100	94	98	94	96

TABLE 22

INACTIVATION OF POLIOVIRUS I BY
TERGISYL AT A CONCENTRATION
OF ONE OUNCE PER GALLON

Sample	pH	Percent Survival Time of Contact Minutes			
		0	10	20	30
Control (No Tergisyl)	7.40	100			79
Tergisyl	9.95	100	100	91	95

INACTIVATION WITH WESCODYNE

Wescodyne, a complexed iodine, was used in an experiment to determine its inactivation efficiency against poliovirus I. Wescodyne, a product of West Chemical Products, Inc., Long Island City, New York, has the following ingredients:

	Percent Present
Polyethoxy polypropoxy polyethoxy ethanol iodine	9.10
Nonylphenoxy polyethoxy ethanol iodine	8.74
Inert ingredients	81.16

The Wescodyne was used in a concentration of 20 mg/l of titrable iodine. The appropriate amount of Wescodyne to be used was determined by titration of the Wescodyne to determine its actual strength. The data for the inactivation are presented in Table 23. The data show that the iodine had an excellent capacity for inactivating the poliovirus with only 0.5 percent surviving after 30 minutes of contact time.

Further work with iodine in a complexed form might be useful since it has been shown to have excellent potential for inactivation of viruses. This work should develop the capacity of the complexed iodine to retain an inactivation potential under the conditions which obtain in the latrine bucket. The ionization products of the complexed form under the various pH and chemical conditions in the pail should be determined.

DISCUSSION OF RESULTS

Little inactivation of the poliovirus type I, Coxsackie A9 and Echo 12 was obtained with a 30 minute contact time and with 10,000 mg/l of the O-D-1435 Type II disinfectant. Contact times of 24 hours with disinfectant at a concentration of 15,000 mg/l permitted a survival of 10 percent of the poliovirus. Virus held under similar conditions for 24 hours but without the disinfectant showed a survival of only 24 percent. The disinfectant increased the inactivation by only 10 percent over the 24 hour exposure period.

Increasing the pH did increase the inactivation obtained but a large portion of this increase was attributed to the pH alone. At a

TABLE 23

INACTIVATION OF POLIOVIRUS I BY WESCODYNE
AT A CONCENTRATION OF 20 MG/L
IODINE

Sample	pH	Percent Survival Time of Contact Minutes			
		0	10	20	30
Control (No iodine)	7.37	100			126
Wescodyne (20 mg/1)	4.14	100	18	2.3	0.5

pH of 12.1 the survival of poliovirus with 10,000 mg/l of disinfectant was less than 0.01 percent after a 5 minute contact period. The survival at this pH without the disinfectant was 58 percent. Clearly these data show a marked potentiation of the disinfectant. As the contact period was extended, the virus survival in the control decreased sharply reaching 6 percent in 20 minutes thus indicating the ability of high pH conditions to inactivate virus. This potentiation of the disinfectant appears to be quite closely related to the pH. The inactivation data at pH 11.9 show only a modest potentiation but at 12.1 the potentiation has increased markedly as indicated above. It would be necessary to increase the pH to a value in excess of 12.0 to obtain this potentiation of the disinfectant. Increasing the pH will increase the amount of inactivation which is obtained. A greater amount of the inactivation which is obtained at a pH above 12 would be caused by the pH effect and a lesser amount from the disinfectant. Consideration must be given, however, to the fact that solutions with these pH values are quite antagonistic to human skin.

Little potentiation of the disinfectant was noted with the surfactants. The cationic and nonionics which were tested were ineffective in potentiating the disinfectant in a concentration of 4,000 mg/l. The expected reaction and precipitation of the disinfectant with these compounds was noted. The sodium stearate, in the maximum soluble concentration of 100 mg/l, decreased the survival of the poliovirus to 74 percent in 30 minutes when it was used alone but decreased the survival with 4,000 mg of the disinfectant to 64 percent. The Neodol 25-3S, an ethoxysulfate, decreased the poliovirus survival with the disinfectant by about 20 percent. Little toxicity to the poliovirus with the straight Neodol 25-3S in concentrations of 100 and 1000 mg/l was noted.

The survival of the poliovirus with 1,000 mg/l of straight Dowfax XD-8214 was 66 percent and was 72 percent with this concentration and 4,000 mg/l of disinfectant. The Dowfax XD-8214 produced an inactivation by itself but did not potentiate the disinfectant so as to produce an increased inactivation. The Dowfax XD-8214 at a concentration of 10,000 mg/l did not produce any inactivation of the poliovirus when used alone or with the disinfectant. The reason for this reversal in behavior at the higher concentration of the surfactant is not known. No inactivation nor potentiation of the disinfectant was noted with the Dowfax 3B2 in concentrations of 100 and 1,000 mg/l. The XD-8214 and the 3B2 are chemically similar with the former having a slighter shorter side chain and less sulfonation. This structural difference was apparently responsible for the difference in the inactivation capacity.

CONCLUSIONS

The following conclusions can be drawn from the results of this work:

1. The O-D-1435 Type II military disinfectant has little capacity for inactivation of viruses when used in concentrations up to 15,000 mg/l with contact times of up to 24 hours.

2. An increase of the pH in excess of 12 will potentiate the disinfectant for poliovirus inactivation. The survival at a pH of 12.1 with 10,000 mg/l of disinfectant was less than 0.01 percent after 5 minutes of contact. The survival at this pH without the disinfectant was 58 percent.

3. The potentiation of the disinfectant for inactivation of poliovirus does not occur until the pH reaches about 12.0.

4. Anionic surfactants have very limited capacity for potentiation of the disinfectant. Cationic and nonionic surfactants had no capacity for potentiation.

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